Claims 71-116 represent the claims canceled, hereby, rewritten in order to address the rejection under 35 USC 112, ¶2, as explained *infra*. Claim 117 defines a method for isolation of putative drug target molecules in a cell.

By virtue of claims 17-117, Applicants replace independent claims 1 and 42 with new claims 71 and 111, respectively. Claims 72-109, directly or indirectly dependent on claim 71, represent claims previously dependent, directly or indirectly, on claim 1. Claim 110 contains the same limitations as in claim 90 (revised claim 22); but, claim 90 is dependent on claim 71, and claim 110 is dependent on claim 72 (corresponding to prior claim 2). Claims 112-116, dependent directly or indirectly on claim 111, represent previous claims dependent on claim 42, directly or indirectly.

Since claims 1 and 70 have been deleted, the rejections of these claims are rendered moot.

Claims were rejected under 35 USC 112, 2nd paragraph. Reconsideration is requested in view of the changes to the claims effected, hereby.

The requisite standard for determining indefinitness under §112, ¶2, is whether one of ordinary skill in the art would be confused as to the meaning of subject matter defined by the language at issue. *In re Kroekel*, 183 USPQ 610 (CCPA 1974). Applying this standard to the claims presented, hereby, demonstrates that the language at issue satisfies the requirements of 35 USC 112, ¶2.

The reasons for the rejection in paragraphs 5 and 6 are addressed by the change of wording to "... wherein each vector in the pool of appropriate vectors in step (a) contains a synthetic totally random DNA sequence ...."

The reasons for the rejection in paragraph 7 are addressed by the amendment of step (e), where it now is stated that the peptides/RNA are expression products from cells exhibiting a changed cellular function/phenotype.

The reasons for the rejection in paragraph 8 has been addressed by deleting step (f) and incorporating the contents thereof in step (e).

The reasons for the rejection in paragraph 9 are addressed by the introduction of the wording "... the biologically active ribonucleic acids or peptides or cellular ligands to the biologically active ribonucleic acids or peptides are identified by ..." before the recitation of step (e).

The reasons for the rejection in paragraph 10 are addressed by the introduction of the amendment helpfully suggested by the Examiner. Applicants respectfully submit that the rejection of claim 15 in this paragraph appears to be erroneous, since the relevant limitation was not recited in claim 15.

Further amendments represented by the instant claims, i.e., of prior claims 7, 8, 15, 16, 17 and 18, address the reasons for the rejection in paragraphs 11-20.

Amendments to claims 20 and 41 (new claims 89 and 102) have been made to bring the claim language in accordance with that of claim 71. This addresses the reasons for the rejection in paragraph 21.

Claim 21 has been limited in scope (new claims 90) and amended to address the reasons for the rejections in paragraphs 22-24. New claim 116 includes the same limitations but is dependent on claim 72 (prior claim 2) and therefore relates to fusions between a polypeptide containing random sequences and a fusion partner.

The amendment of claim 23 (new claim 92) addresses the reasons for the rejection in paragraph 25.

The dependency on claim 71 in claims 94 and 95 (replacing claims 25 and 26) addresses the reasons for the rejections in paragraphs 26 and 27.

Claim 41 has been amended (new claim 102) so as to correspond in wording to claim 71. Thus, the reasons for the rejection in paragraph 28 are addressed.

The amendment of claim 42 (to read "... cells exhibiting up-regulation or down-regulation ...") (new claim 111) addresses the reasons for the rejection in paragraph 29.

Claims 61 and 66 have been amended (new claims 107 and 114) as helpfully suggested by the Examiner and, thus, resolves the reasons for the rejection in paragraph 30. Applicants wish to thank the Examiner for this suggestion, and the suggestion identified above, in order to overcome the corresponding reasons for rejection.

Claims 1-18, 20-26, 30, 31, 37-42, 48, 53 and 59-70 were rejected under 35 USC 103(a) as being unpatentable over WO 95/04824 in view of Labean et al, Dube et al, WO 94/29469, US 5,935,823, Karttunen et al, and von Melchner et al. Reconsideration is respectfully requested.

In an obviousness analysis "all limitations of a claim must be considered in determining the claimed subject matter as is referred to in 35 U.S.C. 103 and it is error to ignore specific limitations distinguishing over the [prior art] reference." *Ex parte Murphy*, 217 USPQ 479, 481 (PO Bd. App. 1982). When, thus, comparing the claims with the prior art, it is the combined teachings of the prior art, taken as a whole, which must be considered in an obviousness analysis. *Ryko Manufacturing Co. v. Nu-Star, Inc.*, 21 USPQ 2d 1053 (Fed. Cir. 1991).

> It is impermissible within the framework of §103 to pick and choose from any one reference only so much of it as will support a given position, to the exclusion of other parts necessary to the full appreciate of what such reference fairly suggests to one of ordinary skill in the art.

In re Hedges, 228 USPQ 685, 687 (Fed. Cir. 1986). The "Examiner bears [both] the initial burden . . . of presenting a *prima facie* case of unpatentability" and "the ultimate burden of persuasion on the issue."

In re Oetiker, 24 USPQ 1443, 1444 and 1447 (Fed. Cir. 1992). "The Examiner can satisfy this burden only by showing some objective teaching in the prior art or that knowledge generally available to one of ordinary skill in the art *would lead* that individual to combine the relevant references. . . . Indeed, the teachings of the references can be combined only if there is some suggestion or incentive to do so." *Ex parte Obukowicz*, 27 USPQ 1063, 1065 (BPA&I 1992)(*emphasis, added*). The "evidence upon which the examiner relies must clearly indicate that a worker of routine skill in this art would view the claimed invention as being obvious." *Ex parte Wolters*, 214 USPQ 735, 736 (BPA&I 1982). To reject a claims for obviousness under §103 based on modifying the teachings of a reference, existence in the prior art of a reason (motivation) to effect the modification is not, by itself, sufficient to sustain the initial burden on the Examiner; the "record" must show

> . . . that it would also have been obvious *how* this [modification] could be achieved . . . . Obviousness . . . must not be judged by hindsight, and a "little modification" can be a most unobvious one.

In re Irani, 166 USPQ 24, 27 (CCPA 1970) (*emphasis in original*).

Keeping the foregoing standards of obviousness in mind, the rejection is traversed, first, concerning applying the cited references cited against the subject matter of the instant independent claims.

WO 95/04824: As correctly explained in the statement of rejection, WO 95/04824 discloses a method of transducing eukaryotic cells with a viral vector, which stably integrates into the genome of a cell at one to a few copies per cell expressing an encoded sequence. However, contrary to the opinion expressed in the statement of rejection, WO 95/04824 does not disclose that the encoded sequence may be totally random.

Rather, it is indicated that up to 100% of the *pool* of encoded sequences may consist of randomized sequences, cf. Page 3, lines 6-7 in WO 95/04824. There is, however, no direct teaching when it comes to the degree of randomization in *each sequence* in the pool. It is stated in Example 16: "cDNA libraries carrying cDNA molecules are generated using the high error rate PCR procedure of Caldwell and Joyce (34)"; citation "(34)" being identified in WO'824 (page 26) as *PCR Methods and Applications 2*, 28-33 (1992). Similar reference to Caldwell and Joyce is made on WO'824 at page 3, line 6. Hence, when discussing mutations, WO 95/04824 relies exclusively on Caldwell and Joyce. For the convenience of the Examiner, a copy of the Caldwell and Joyce reference is attached, hereto.

Upon the optimum conditions defined by Caldwell and Joyce (cf. the attached copy), error prone PCR results in a mutagenesis rate of 0.66% per base per PCR (30 cycles) (page 30, left column, first paragraph and Table 1). Accordingly, this mutation rate corresponds to approximately 1 mutation per 152 nucleotides per PCR. Thus, following error prone PCR, the vast majority of a DNA

sequence remains unchanged (page 30, Fig. 2) and is closely based on the starting DNA sequence. It therefore cannot be said to be randomized in the sense used in the description and claims of the present application which requires the use of *synthetic totally random DNA*. Moreover, Caldwell and Joyce demonstrate that attempts to increase the mutation rate results in a bias for changes involving A and T residues in comparison to changes in G and C residues by error prone PCR, which further precludes randomization of a cDNA sequence (page 29, right column, paragraph 2, and page 30, Table 1).

Thus, high error rate PCR cannot give rise to "synthetic completely random DNA sequences" as presently claimed. Rather, each DNA product obtained when using high error rate PCR only differs in one out of 152 bases compared to the parent DNA sequence. The mutation rate in polypeptides encoded by such high error rate PCR products will be less than 1/152, since the degeneracy of the genetic code will render a substantial fraction of such mutations silent at the protein level (one-third of all mutations will hit the last base in a codon, and of these some two-thirds does not result in a change in expression product - thus, at least 22% of all mutations introduced via high-error rate PCR are silent mutations).

Hence, the inevitable teaching of WO 95/04824 is that the DNA inserted into retroviral vectors and subsequently expressed in eukaryotic cells must be DNA with a small variation compared to the original sequences. Accordingly, the use of the vectors for expression purposes must give rise to an expression library where only small changes in the expression products are present.

Dube et al: With respect to Dube et al, the statement of rejection maintains this reference teaches "... the method of using totally or partially random sequences in a viral vector to transform a eukaryotic cell ...." Applicants have studied Dube et al carefully and Applicants have not been able to identify one single passage which relates to transformation of eukaryotic cells. All experiments reported utilize prokaryotic cells (*E. coli*).

Labean et al.: In essence, Labean et al. deals with the problems involved in producing suitably biased random DNA libraries in order to render the expression products thereof longer and free of stop codons. There is, however, no mention in this reference that such DNA libraries would be useful for transducing eukaryotic cells in order to identify biologically active peptides or RNA, let alone to isolate cellular peptide ligands to such biologically active peptides or RNA.

U.S. 5,935,823: Concerning U.S. 5,935,823, the reference teaches a method for preparing Totally Synthetic Affinity Reagents (TSARs). The method comprises preparation of DNA encoding TSARs consisting of fusions between a putative ligand binding domain and a biologically active part. The TSAR encoding sequences are used to transform cells to express the fusion proteins and after that the fusion proteins are used to screen for binding to *a ligand of choice*, cf. Column 13, lines 5-7.

Consequently, U.S. 5,935,832 does not include a screening for phenotypic changes in the transformed cells as claimed in present claim 71.

The tenor of the statement of rejection's obviousness rejection seems to be that the skilled person would readily substitute the cDNA of WO 95/04824 with randomized DNA known from Labean et al, Dube et al and U.S. 5,935,823.

As detailed above, WO 95/04824 teaches use of libraries of randomly mutagenized cDNA where about 1 out of 152 bases in each PCR generated cDNA molecule of the library has been mutated. This means that the expression products of the mutagenized cDNA has a high sequence identity with the parent cDNA. This must, in applicants' opinion, have the implication that use of the expression cloning method of WO 95/04824, when applied on DNA mutants, only allows for discovery of mutants of the parent cDNA which encode variants with an activity comparable to the expression product of the known parent cDNA. Or, phrased otherwise, expression cloning typically relies on a cellular detection system, where one screens for an equivalent of an expression product of a known cDNA fragment (e.g., by using a cell where the gene encoding the known cDNA has been "knocked out" or is naturally absent and the introduction of an equivalent replaces the natural function of the known cDNA). It would certainly not be feasible to use the system of WO 95/04824 to utilize cDNA and mutants thereof in an attempt to find new interactions in cells.

Applicants can find no suggestions in the cited prior art references that it would be feasible, let alone desirable, to use a library of *synthetic totally random DNA* as presently claimed in order to search for *in vivo* biologically active peptides/RNA - in fact, none of the cited references teach or suggest that such short peptides would be likely to have a detectable effect *in vivo*. The members of a library as defined in the present invention are not related in sequence to any parent DNA sequence, and they are (due to the use of complete randomization) inherently very short. None of the cited references address the question of whether short peptides would at all be sufficiently stable so as to be able to interfere with the biochemistry of a cell thereby producing a detectable phenotypic change.

There is also a profound and inherent difference between the information one can obtain form the expression cloning techniques disclosed in WO 95/04824 and the information which is obtained by utilizing the presently claimed invention. The teaching of WO 95/04824 allows for identification of large molecules with a biological function -even if such a molecule should (by chance) exert its biological function through interaction with target molecules in the transduced cell, ti would not be possible to conclude anything about the nature of this interaction and the expression product could never function as a putative lead compound in drug development and would not be of help in identifying the target molecules (since a large molecule, due to its size, will bind to many different molecules in a cell).

Contrary to this, the present invention provides as a result that the short random expression product from a cell exhibiting altered characteristics after transduction most likely encodes a peptide/RNA which interferes directly with a target molecule in the transduced cell. And, due to its small size, the random expression product can serve as a drug lead compound and be used for isolation of the target molecule in the cell.

The statement of rejection has not provided any indication of teachings in the cited references which would prompt the skilled person of the advantage of substituting the long DNA fragments in WO 95/04824 with short, completely random DNA sequences. The three secondary references all deal with the use of randomized fragments in drug discovery methods where a known target molecule is used to fish out random peptides from a library. Such known methods require access to the target molecule, but also makes it possible to optimize the screening procedure by providing large amounts of random peptides and of target molecule. As an example, when performing screenings using phage display, the skilled person has full control over the reaction conditions between the phage library members and the known target molecule. This is not the case in the present invention, where neither the concentrations of the library expression product nor the (unknown) target molecule are known.

However, to conceive the present invention it would have to be accepted by the skilled artisan that it would be possible to obtain a biological effect in a cell which is transduced with a short random fragment. It is not suggested in any of the cited references that such a short random fragment could 1) be expressed by a viral vector and 2) exhibit a biological detectable effect in a cell.

In summary, therefore:

First, exercise of the method of WO 95/04824 gives rise to a completely different set of information concerning library members than does the presently claimed invention and the method of WO 95/04824 cannot provide for identifying putative drug target molecules as presently claimed in claim 72. None of the cited secondary references suggests that advantageous information could be obtained in the system of WO 95/04824, when using completely random synthetic DNA, because use of short fragments of DNA would not be able to achieve the goals obtained by using DNA.

Second, there is no teaching or suggestion in the prior art references that would have led the skilled artisan to expect that a library of short completely random DNA fragments would be capable of functioning in a screening as presently claimed. All prior art references have relied on screening systems that include full control of the concentrations of a known target ligand and, possibly also, of library members. Such a control cannot exist in the presently claimed method; and, therefore, the skilled person would not have seriously even contemplated, let alone considered obvious, a variation of the expression cloning system of WO 95/04824 using synthetic, completely random sequences.

For the foregoing reasons, Applicants submit that the skilled person would have found no motivation to combine WO 95/04824 with any of the three secondary prior art references discussed above.

Concerning the secondary references Karttunen et al and von Melchner et al, their teachings are relied upon in the statement of rejection for reasons that are not relevant to the defects in the other cited references, as explained above. As such, neither Karttunen et al nor von Melchner et al, taken together or separately, cure the fatal deficiencies in the other references relied upon in alleging the claimed invention would have been obvious under §103 of the statute.

Favorable action is requested.

Respectfully submitted,

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